2nd International Citrus Canker and Huanglongbing Research Workshop

Huanglongbing

- **Research Recommendations – HLB**
- **HLB Priority Research Recommendation Chart 2005**
- **HLB Priority Prioritized Research Recommendation List**

Citrus Canker

- **Research Recommendations – Citrus Canker**
- **Citrus Canker Priority Research Recommendation Chart 2005**
- **Citrus Canker Prioritized Research Recommendation List**

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Research Recommendations: Huanglongbing 2005

- Economics
- Alternate Hosts
- Detection of Disease/Vector
- Differentiation
- Resistance and Breeding
- Culturing HLB
- Pathogen/Vector Interactions
- Citrus Genetics
- Chemical Control
- Biological Control
- Cultural Control
- Pathogenesis
- Epidemiology
- Transgenics
- Genomics
- Vector Biology
- Fruit Yield/Quality

T.R. Gottwald, J. Graham, W.N., Dixon, A. LaVigne and P. Berger. 2005. Proceedings of the 2nd International Citrus Canker and Huanglongbing Research Workshop. Orlando, FL.

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1 Economics

1.1 Economic Analysis

- Economic losses due to restricted movement
- Economic analysis of lost markets (domestic and international)
- Economic benefit of tree removal
- Economic analysis of control measures
- Phytosanitary systems for fruit movement from quarantine areas
- Rotational/cropping systems
- Ornamental industry
- Pest risk analyses
- Phytosanitary measures for nurseries

Research Recommendations: Huanglongbing 2005

2 Alternative Hosts

- 2.1 Identify alternative hosts of pathogen/vector and geographic distribution
- 2.2 Bacterial population level in alternative hosts
- 2.3 Risk assessment of alternative hosts
- 2.4 Vector biology ref. alernative hosts

3 Detection of Disease and Vector

- 3.1 Field vs Nursery Symptomology
- 3.2 Development Of New Serological Tools
- 3.3 EM, Light Microscopy, CF
- 3.4 Molecular Detection Methods
  - Dot blot probes (vectors)
  - PCR – 16S RNA; rpl-based PCR; Duplex: nested PCR; Multiplex Real-time PCR
  - Improve detection (sensitivity); understand host-pathogen interaction leading to better control, etc.
- 3.5 Sentinel Indicator Plant
- 3.6 Sample Criteria
- 3.7 Psyllid Population Density Sampling
- 3.8 Chemical or Volatile Detection
- 3.9 Storage of Samples Effects
- 3.10 Remote Sensing

- 4 Characterization Taxonomy of HLB
  - 4.1 Differentiation of species and strains
  - 4.2 Genetic diversity studies
  - 4.3 Repositories for voucher specimens

- 5 Resistance and Breeding
  - 5.1 Assessment of Citrus Relatives For Resistance/Susceptibility
  - 5.2 Cold Hardiness for Citrus
  - 5.3 Host Genotype Strain/Species Interactions
  - 5.4 Vector Repellency/Lethal Genes
  - 5.5 Field Testing of Cultivars

- 6 Culturing HLB
  - 6.1 Culturing HLB

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- 7 Pathogen/Vector/Host Interactions
  - 7.1 Population In Different Cultivars
  - 7.2 Pathogen Vector Relationships
    - Transovarial Transmission

- 8 Chemical Control
  - 8.1 Insecticides To Keep Psyllids Off Nursery And Grove Bearing And Non-bearing Trees
    - Scouts
    - Encourage Beneficials
  - 8.2 Effect of Insecticides on Disease Spread
    - Duration of Protection
  - 8.3 Urban Homeowner Control of Psyllids
  - 8.4 Systemic Bactericide
  - 8.5 Baseline Toxicity Studies
  - 8.6 Natural Chemicals
  - 8.7 Application Methods and Interaction With Beneficials
  - 8.8 Chemicals to Control Flush
  - 8.9 Effect of Toxicants On Vector Transmission

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- 9 Biological Control
  - 9.1 Determination of Presence of Hyperparasites
  - 9.2 Foreign Exploration
  - 9.3 Trap Plants (Mp Et Al)
  - 9.4 Biological Control of Pathogen
  - 9.5 BC of Vector in Residential Areas
  - 9.6 Distribution of Parasitoids and Efficiency

- 10 Cultural Control
  - 10.1 HLB-free budwood
  - 10.2 Nursery design, management and location away
    - Budwood sources/nurseries under screen
  - 10.3 Pruning and rogueing
  - 10.4 Orchard design and management
    - Intercropping
  - 10.5 Greenhouse production of citrus
  - 10.6 Cultural control of alternate hosts

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- 11 Epidemiology
  - 11.1 Invasive Potential of Disease And Vector
  - 11.2 Effect of Cultural Practices
    - Effect Of Effect of Vector Control on Disease Development
    - Rogueing
    - Effect of Trap Plants
  - 11.3 Effect of Insect Population Dynamics on Disease Dynamics
  - 11.4 Distance of Disease and Vector Spread
  - 11.5 Aging Infection
  - 11.6 Seed Transmission and Graft Transmission from Asymptomatic Plants
  - 11.7 Proportion of Infected Insects in Population Relative to Disease Incidence
  - 11.8 Survey of Incidence and Distribution in FL
  - 11.9 Eradication Methods

12 Transgenics

- 12.1 Find and introduce resistance:
- 12.2 Resistance genes in related spp.
  - Anti-bacterial genes
  - Anti-bacterial peptides
  - Use of viral vectors
  - Anti-insect genes
- 12.3 Rapid screening method for resistance
- 12.4Genetic modification of vector
- 12.5 Development of transformation methodologies for citrus and citrus relatives and ornamentals

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- 13 Genomics
  - 13.1 Citrus Responsive Genes for Early Detection
  - 13.2 Sequencing of Bacterial Genomes
  - 13.3 Sequencing of Citrus Genome
  - 13.4 EST Microarray
  - 13.5 Comparative and Functional Genomics

- 14 Vector Biology
  - 14.1 Reproductive Biology And Behavior
  - 14.2 Pheromones and Attractants
  - 14.3 Dispersal Behavior of Vector

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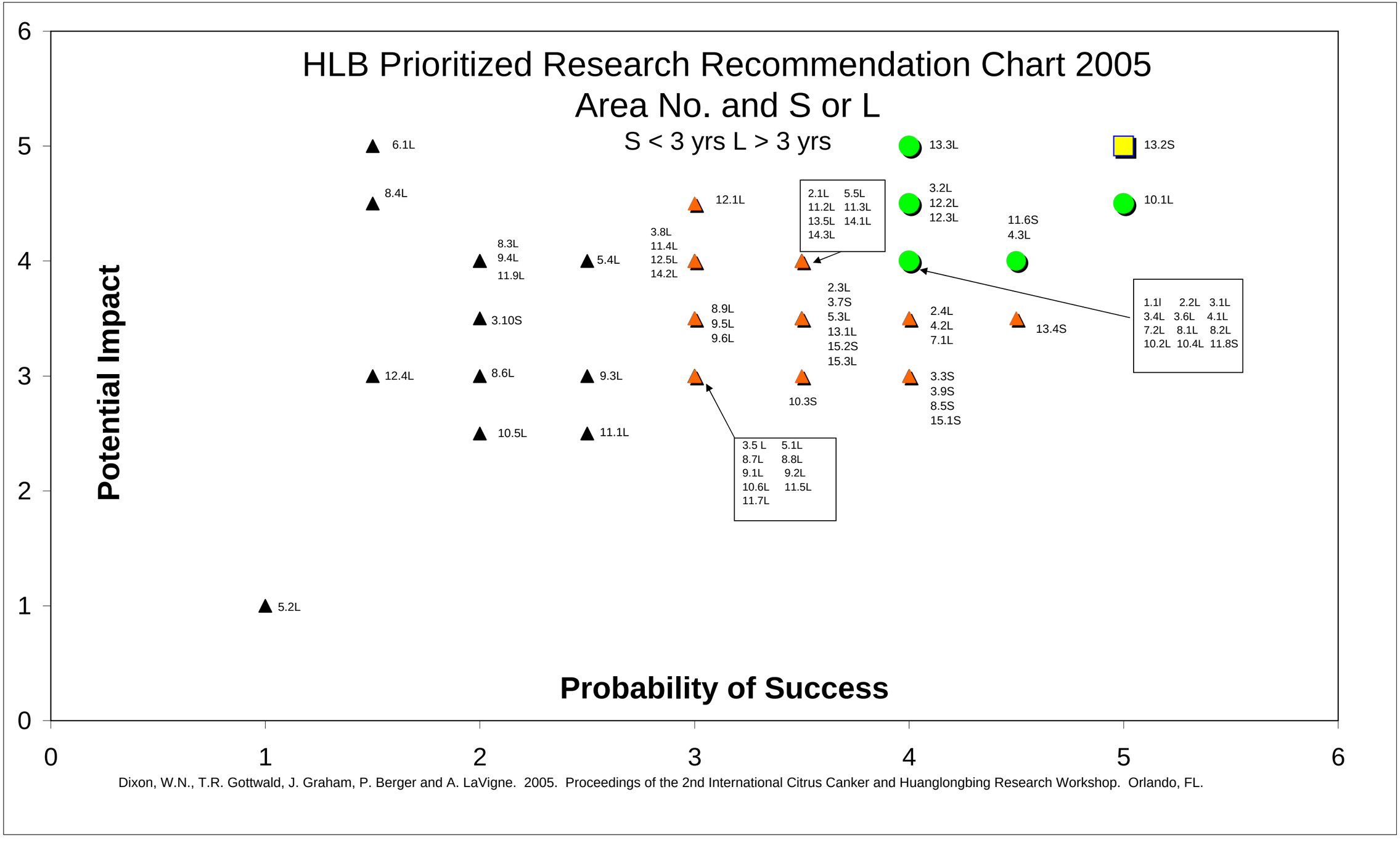
- 15 Fruit Yield and Quality
  - 15.1 Relationship of Fruit Quality to Disease Incidence
  - 15.2 Physical Means for Culling
  - 15.3 Crop or Yield Loss Models



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Huanglongbing Prioritized Research Recommendation List

Scale is 1 (low) to 5 (high)

Area No.	Project	Short Term < 3yrs	Long Term > 3 yrs	Probability of Success	Potential Impact
1	**Economics**				
	1.1		x	4	4
2	**Alternative Hosts**				
	2.1		x	3.5	4
	2.2		x	4	4
	2.3		x	3.5	3.5
	2.4		x	4	3.5
3	**Detection of Disease and Vector**				
	3.1	x		4	4
	3.2		x	4	4.5
	3.3	x		4	3
	3.4		x	4	4
	3.5		x	3	3
	3.6	x		4	4
	3.7	x		3.5	3.5
	3.8		x	3	4
	3.9	x		4	3
	3.10		x	2	3.5
4	**Characterization and Taxonomy**				
	4.1		x	4	4
	4.2		x	4	3.5
	4.3		x	4.5	4
5	**Resistance and Breeding**				
	5.1		x	3	3
	5.2		x	1	1
	5.3		x	3.5	3.5
	5.4		x	2.5	4
	5.5		x	3.5	4
6	**Culturing HLB**				
	6.1		x	1.5	5
7	**Pathogens/Vector/Host Interactions**				
	7.1		x	4	3.5
	7.2		x	4	4
8	**Chemical Control**				
	8.1		x	4	4
	8.2		x	4	4
	8.3		x	2	4
	8.4		x	1.5	4.5
	8.5	x		4	3
	8.6		x	2	3
	8.7		x	3	3
	8.8		x	3	3
	8.9		x	3	3.5
9	**Biological Control**				
	9.1		x	3	3
	9.2		x	3	3
	9.3		x	2.5	3
	9.4		x	2	4
	9.5		x	3	3.5
	9.6		x	3	3.5
10	**Cultural Control**				
	10.1		x	5	4.5
	10.2		x	4	4
	10.3	x		3.5	3
	10.4		x	4	4
	10.5		x	2	2.5
	10.6		x	3	3
11	**Epidemiology**				
	11.1		x	2.5	2.5
	11.2		x	3.5	4
	11.3		x	3.5	4
	11.4		x	3	4
	11.5		x	3	3
	11.6	x		4.5	4
	11.7		x	3	3
	11.8	x		4	4
	11.9		x	2	4
12	**Transgenics**				
	12.1		x	3	4.5
	12.2		x	3	4.5
	12.3		x	3	4.5
	12.4		x	1.5	3
	12.5		x	3	4
13	Genomics				
	13.1		x	3.5	3.5
	13.2	x		5	5
	13.3		x	4	5
	13.4	x		4.5	3.5
	13.5		x	3.5	4
14	Vector Biology				
	14.1		x	3.5	4
	14.2		x	3	4
	14.3		x	3.5	4
15	Fruit Yield and Quality				
	15.1	x		4	3
	15.2	x		3.5	3.5
	15.3		x	3.5	3.5

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Research Recommendations: Citrus Canker 2005

- Remote Sensing
- Spectral Analysis
- Economics
- Survival of Xac
- Detection of Disease
- Differentiation
- Resistance
- Citrus Breeding
- Citrus Genetics
- Citrus Resistance
- Chemical Control
- Biological Control
- Cultural Control
- Pathogenesis
- Epidemiology
- Transgenics
- Genomics

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Research Recommendations: Citrus Canker 2005

- 1 Chemical
  - 1.1 Evaluation of ISR/SAR etc. related to ccA
  - 1.2 Application Methods for Chemicals – Aircraft et al.
  - 1.3 Investigation of Curative and Preventative Properties of Microbicides
  - 1.4 Combinations of Chemical Controls with Copper – IPM
  - 1.5 Asian Citrus Leaf Miner
    - Evaluation of New and Existing Compounds: Vydate, E2Y, Copper GX
    - Pheromones monitoring and mating disruption
  - 1.6 Resistance of Xac to copper
  - 1.7 Testing of sanitizing compounds to pre- post-harvest, packing house
  - 1.8 Search for systemic bactericide

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- 2 Cultural Control
  - 2.1 Effect of Irrigation and Spray Practices on Disease Increase
  - 2.2 Optimization of Windbreaks
  - 2.3 Pruning (disease control, dwarfing)
  - 2.4 Field Susceptibility of Cultivars - Flush management
  - 2.5 Nutrition
  - 2.6 Defoliation
  - 2.7 Orchard Management Systems
  - 2.8 Mechanical Harvesting Impact
  - 2.9 Protected Production (relocation, greenhouses: nursery vs grove)
  - 2.10 Alternative Land Uses during Fallow Period
  - 2.11 Investigation of Practices – foreign sources

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- 3 Biological Control
  - 3.1 Xanthomonas Control with Bacteriophages to Decrease Inoculum
  - 3.2 Use of Antagonistic or Site-competitive Microorganisms
  - 3.3 Interaction of A and B Strains of Citrus Canker and Their Competition
  - 3.4 Microbial Community Phyloplane and Endophytes
  - 3.5 Citrus Leaf Miner
  - 3.6 Cross-protection using Avirulent Canker Strains
  - 3.7 Antangonistic Effects of HLB Controls

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- 4 Remote Sensing and Information/Tracking (GIS+) Systems
  - 4.1 Proof of Concept of Spectral Analysis to Citrus Canker
  - 4.2 Application and Deployment
    - Low Level spectral Characteristics
    - Application to Finding Citrus Canker / Citrus Trees
    - Focus on Aircraft-based Hyperspectral Analysis

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- 5 Detection Technology
  - 5.1 Prove Canines can Differentiate Citrus Canker
  - 5.2 Visual Detection – Sensitivity & Reliability
  - 5.3 Electronic Noses - Pathways for Citrus Entry
  - 5.4 Electronic Noses - Application to Citrus and Citrus Canker Detection
  - 5.5 Quantitative PCR to Detect Non-culturable Citrus Canker
  - 5.6 Detect Host Response prior to Lesion Development – microarrays et al.
  - 5.7 High Throughput of PCR for Citrus Canker Detection
  - 5.8 Improved Detection Sampling Designs
  - 5.9 Nanotechnology
  - 5.10 Field Deployable Rapid Detection Technology
  - 5.11 Nursery Detection Technology

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Research Recommendations: Citrus Canker 2005

- 6 Citrus Resistance and Breeding
  - 6.1 Knowledge of Pathogen-based Resistance
  - 6.2 Genomic Comparisons - Resistance Responses
  - 6.3 Citrus Resistance Triggers and Map-based Cloning
  - 6.4 Generation of Resistant Germplasm
  - 6.5 Performance of Resistant Cultivars from Worldwide Sources
  - 6.6 Genetic Characterizations of Resistance in Citrus
  - 6.7 Rutaceae Susceptibility to Citrus Canker
  - 6.8 High Throughput/Improved Screening Systems
  - 6.9 Determination Of Biological Elicitors of Plant Defenses
  - 6.10 Lytic Peptides and Delivery Systems
  - 6.11 Develop Markers For Selection In Breeding Programs Linked to Resistance
  - 6.12 Differentially Expressed Genes - cca EST or cdna Library
  - 6.13 Exploitation Of Resistance Gene Candidate Sequences Already Cloned from Citrus
  - 6.14 Classical Breeding Techniques for Resistance
  - 6.15 Dwarfing Rootstocks
  - 6.16 Foreign Exploration for Other Sources
  - 6.17 Expedited Field Trials for Performance

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Research Recommendations: Citrus Canker 2005

- 7 Differentiation / Characterization of Xac Strains
  - 7.1 Standardization and Quality Assurance:
    - Ring test and methods of certification; global web site
  - 7.2 Establish International Collections / Repositories
    - Permanent Florida, national (Beltsville), and international location
    - Funding and collection size
  - 7.3 Improve Rapid Strain Differentiation Techniques
  - 7.4 Strain Characterization for Origin
    - Differentiation for host/pathogen interactions
    - Creating marked strains

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- 8 Pathogenesis
  - 8.1 Nature of Mesophyll Resistance to ccA
  - 8.2 Xanthomonas Genomics and Functional Analysis

    Identification of genes necessary for

    1) infection and

    2) induction of resistance expression due to infection

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- 9 Survival
  - 9.1 Survival of Bacterium in Packing Container
  - 9.2 Probability of Transmission from Fruit and or Plant Materials Disinfested
  - 9.3 Survival of Bacterium on Lesioned or Lesionless Plant Tissues
  - 9.4 Use Dilution Strength, Biodegradable, Bacteriocide

    Develop all-purpose disinfectant

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- 10 Economics
  - 10.1 Economic Analysis
    - Economic losses due to restricted movement
    - Conclusive science to achieve a defensible position related to risk of fruit movement (risk assessment: Florida's white paper, in part)
    - Economic analysis of lost markets (domestic and international)
    - Economic benefit of defoliation vs tree removal
    - Economic analysis of control measures
    - Phytosanitary systems for fruit movement from quarantine areas

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- 11 Transgenics
  - 11.1 Differentially Expressed Libraries to Identify Promoters
  - 11.2 Transgenic Citrus with Resistance Genes from Citrus and other Plants and Organisms
  - 11.3 Transformation System Development
  - 11.4 High throughput Screening
  - 11.5 Transgenic Rootstocks
  - 11.6 Use of Viral Vectors
  - 11.7 Interaction between Scion and Rootstock
  - 11.8 Site-directed Mutagenesis
  - 11.9 Novel Technologies

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- 12 Genomics
  - 12.1 Differentially Expressed Libraries in Response to Asian Citrus Leaf Miner feeding
  - 12.2 Sequencing citrus genome

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- 13 Epidemiology
  - 13.1 International Field-scale Study (multinational):
    - Large vs small scale
    - Sampling Methods / Technology
    - Visual survey efficiency
    - Deployment of survey and sampling technologies (coordination)
    - Chemical of leafminer and Xac, windbreaks, weather forecast systems, defoliation, irrigation etc.
  - 13.2 Meteorological Events and their Distance of Spread
    - Effects on development of disease
    - Evaluation in Different Cultural Settings
    - Local, international, greenhouse / laboratory
  - 13.3 Latency Duration of Fallow
  - 13.4 Isolation Distances for Nurseries
  - 13.5 Alternative Distances and Timing

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13. 6 Control studies within an Endemic / Epidemic

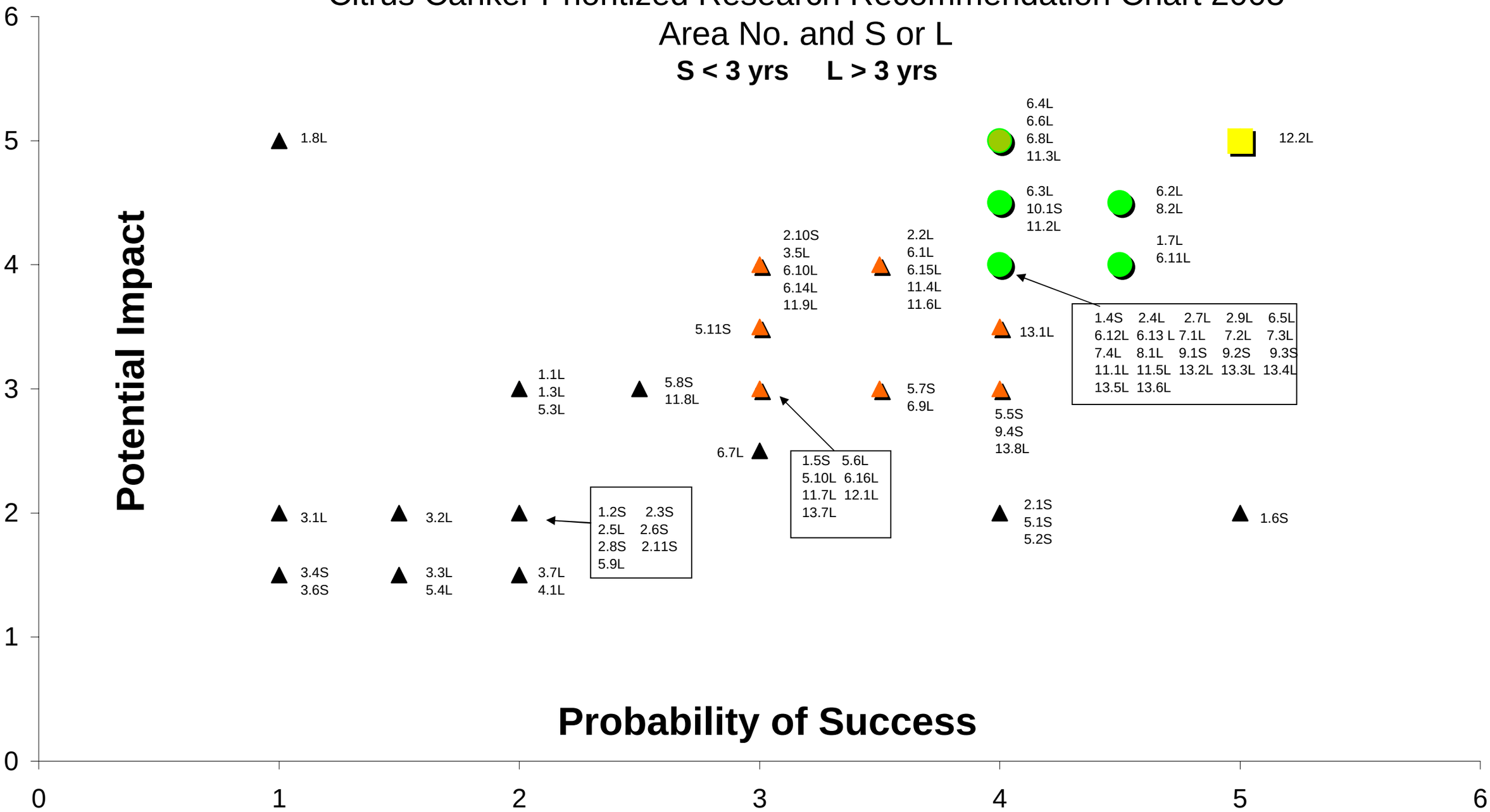
- Eradication Campaign – Epidemic
- Management – Endemic: surrogate organisms, environmental variations
- Application and impact of windbreaks, defoliation techniques
- Pre-eradication inoculum suppression techniques – defoliation, tarping

- 13.7 Insecticide / Microbicide / Surfactant Influences
  - Enhance disease expression on trap plants using surfactants
  - Cuticle studies for adjuvants and penetrants for systemic chemical delivery
  - Microbicide as prevention of inoculum transfer using local or systemic compounds
- 13.7 Damage Evaluation System

Citrus Canker Prioritized Research Recommendation Chart 2005

Area No. and S or L

S < 3 yrs L > 3 yrs



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Citrus Canker Prioritized Research Recommendation List

Scale is 1 (low) to 5 (high)

Area No.	Project	Short Term < 3yrs	Long Term > 3 yrs	Probability of Success	Potential Impact
1	**Chemical**				
	1.1		x	2	3
	1.2	x		2	2
	1.3		x	2	3
	1.4	x		4	4
	1.5	x		3	3
	1.6	x		5	2
	1.7	x		4.5	4
	1.8		x	1	5
2	**Cultural Control**				
	2.1	x		4	2
	2.2		x	3.5	4
	2.3	x		2	2
	2.4		x	4	4
	2.5		x	2	2
	2.6	x		2	2
	2.7		x	4	4
	2.8	x		2	2
	2.9		x	4	4
	2.10		x	3	4
	2.11	x		2	2
3	**Biological Control**				
	3.1		x	1	2
	3.2		x	1.5	2
	3.3		x	1.5	1.5
	3.4		x	1	1.5
	3.5		x	3	4
	3.6		x	1	1.5
	3.7		x	2	1.5
4	**Remote Sensing**				
	4.1		x	2	1.5
	4.2		x	1.5	2
5	**Detection**				
	5.1	x		4	2
	5.2	x		4	2
	5.3		x	2	3
	5.4		x	1.5	1.5
	5.5	x		4	3
	5.6		x	3	3
	5.7	x		3.5	3
	5.8	x		2.5	3
	5.9		x	2	2
	5.10		x	3	3
	5.11	x		3	3.5
6	**Resistance**				
	6.1		x	3.5	4
	6.2		x	4.5	4.5
	6.3		x	4	4.5
	6.4		x	4	5
	6.5		x	4	4
	6.6		x	4	5
	6.7		x	3	2.5
	6.8		x	4	5
	6.9		x	3.5	3
	6.10		x	3	4
	6.11		x	4.5	4
	6.12		x	4	4
	6.13		x	4	4
	6.14		x	3	4
	6.15		x	3.5	4
	6.16		x	3	3
	6.17		x	4	4
7	**Differentiation**				
	7.1		x	4	4
	7.2		x	4	4
	7.3		x	4	4
	7.4		x	4	4
8	**Pathogenesis**				
	8.1		x	4	4
	8.2		x	4.5	4.5
9	**Survival**				
	9.1	x		4	4
	9.2	x		4	4
	9.3	x		4	4
	9.4	x		4	3
10	**Economics**				
	10.1	x		4	4.5
11	**Transgenics**				
	111		x	4	4
	11.2		x	4	4.5
	11.3		x	4	5
	11.4		x	3.5	4
	11.5		x	4	4
	11.6		x	3.5	4
	11.7		x	3	3
	11.8		x	2.5	3
	11.9		x	3	4
12	**Genomics**				
	12.1		x	3	3
	12.2		x	5	5
13	**Epidemiology**				
	13.1		x	4	3.5
	13.2		x	4	4
	13.3		x	4	4
	13.4		x	4	4
	13.5		x	4	4
	13.6		x	4	4
	13.7		x	3	3
	13.8		x	4	3

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